E. 4 Expression of His consructs

28.07.2014

Anna, Oli, Melanie

SDS-Page of Constructs B, C, X, Y for Western Blot (all Blue Marker) and Coomassie Staining (unstained marker)

Pouring of a 15 % SDS-Gel

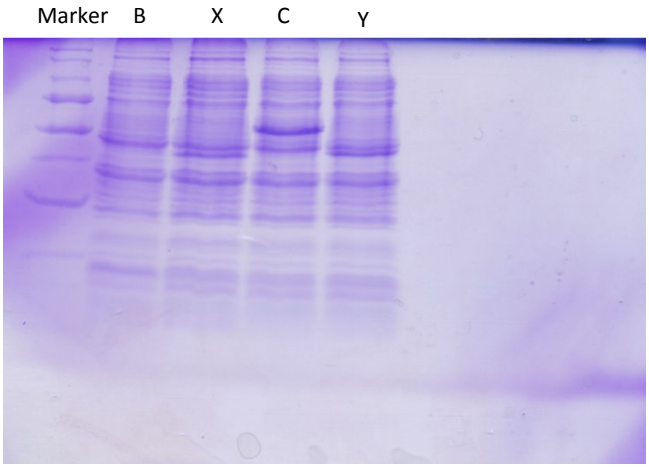
Loading of 2 SDS-Gels with in one case all 10 µL of all Blue Marker for the Western Blot and in the other with Unstained Marker for Coomassie-Staining (+ 10 µL each construct)

Starting program P1 with 200 V for 44 min.

Coomassie gel:

Staining: Coomassie-briliant blue in 10% acetic acid: for 45 min on shaker

Destaining: 45 min in 10% acetig acid on shaker

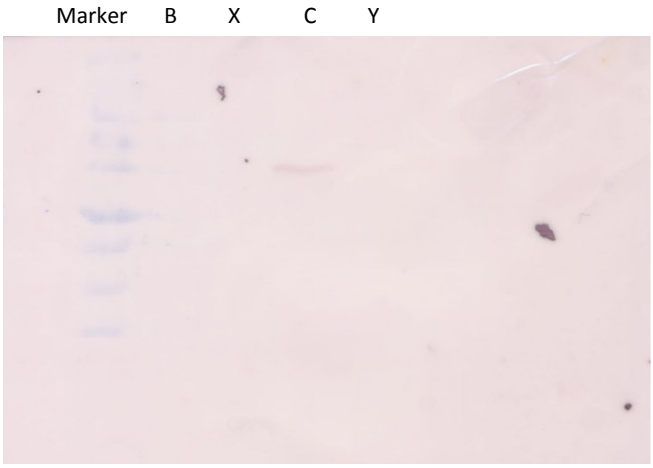


Immunostain gel:

Dying with snap id:

1. 15 min blocking in 30 mL 0,05 % MPBS
2. 3x washing with 1x PBS
3. Incubation with 3 mL anti-His antibody (Qiagen anti-His, mouse, dilution 1:5000 (because snap id was used))
4. 3x washing with 1x PBS
5. Incubation with 3 mL anti-mouse-AP (Dianova, AP-conjugated, dilution 1:1000)
6. 3x washing with 1x PBS

100 mL AP-substrate-buffer with 100 µL BZIP and 100 µL NTB. Incubation for 20 min.



- Only one band is visible in the mmoC sample but it is around 37 kDa instead of the expected 61,5 kDa of mmoX

07.08.2014

Niels, Steffen, Maren, Christian

1. Over Night Cultures 50 mL 2xYT-Ca-Glucose inoculate from GlyStock, OD ≈0,1

2. Change of Medium

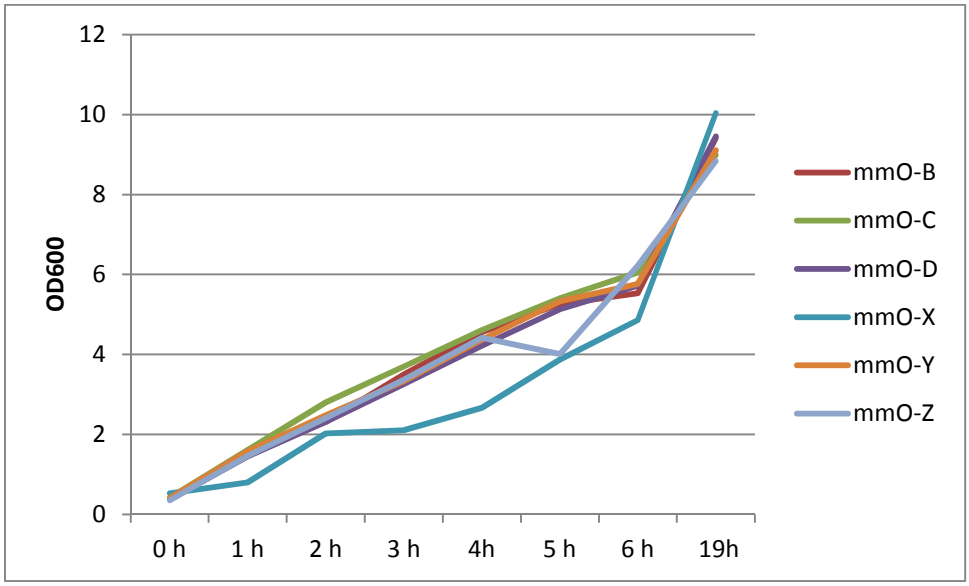
Centrifuge each suspension for 10 min(full speed, 4x 50 ml suspension in 50 ml falcon tube), resuspend the pellet in 50ml -Ca-Glucose-Medium with 100 µmol IPTG

Merge contents for each construct (200ml) and cultivate at 37° C, 250 rpm.

a) Measure OD_{600} and centrifuge 1 mL, store pellet and supernatant in -20°C every hour for 6 hours

Attention: Starting at 2h: Only 900 µl were harvested!

OD_{600}	0 h 14:45	1 h	2 h	3 h	4h	5 h	6 h	Next day 9:15
mmO-B	0,39	1,497	2,31	3,5	4,55	5,26	5,53	9,41
mmO-C	0,416	1,61	2,8	3,7	4,61	5,41	6,05	8,99
mmO-D	0,373	1,446	2,31	3,26	4,22	5,13	5,72	9,45
mmO-X	0,528	0,8	2,02	2,1	2,66	3,87	4,86	10,04
mmO-Y	0,406	1,572	2,48	3,33	4,37	5,33	5,76	9,11
mmO-Z	0,353	1,46	2,41	3,37	4,42	4,01	6,22	8,84



08.08.2014

Maren, Oli

Harvest the rest of each suspension in one 50 ml Falcon by centrifugation and store at -20°C.

11.08.2014

Nils, Maren

15% SDS-Page of Samples 0h, 3h, 6h and 19 hours after Induction with IPTG

Resuspend samples in 1x Laemmli in PBS to OD_{600} = 0,1

And mmO-D 3h [Pellet of 0,9ml]: 1,446 -> resuspend in (1,446·90=)293,4 µl 1x Laemmli-PBS)

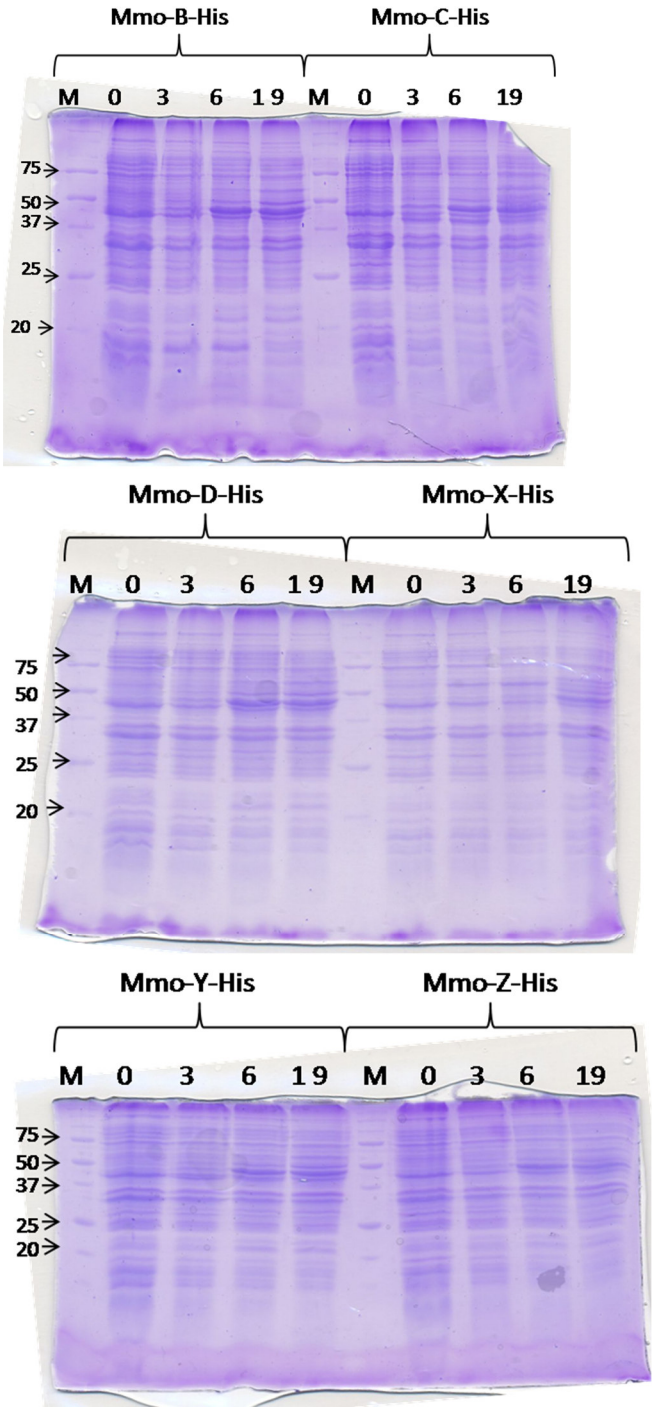


Fig. 1 SDS-Page of His-constructs, 0= at point of induction with IPTG, 3= 3 h after induction, 6= 6 h after induction, 19= 19h after induction

13.8.14

Maren, Lukas

Analysis of His-Expression

- Resuspend pellet of rest of solution after 19 h after induction (see 8.8.14) in 5ml PBS

- Disruption by sonfication (1 min, 60%, MS73)

- centrifuge 15 min, 800g, 4°C

- Supernatant: Transfer into new Falcon, Zentrifuge for 30 min, 6000g, 4°C
  Supernatant: soluble Fraction
- Pellet: Resuspend in 10 ml PBS, Zentrifuge for 15 min, 3000g, 4°C
  Discard supernatant, resuspend pellet in 10 ml PBS, Zentrifuge for 15 min, 6000g, 4°C
  Discard supernatant, resuspend pellet in 2 ml PBS-8m Urea
  Transfer into 2 ml Eppi, Zentrifuge for 20 min, 11.000g, 4°C
  Transfer supernatant into new 2ml Eppi: Inclusion Fraction

SDS-PAGE

Mix 80µl of each sample (soluble fraction and inclusion fraction) + 20 µl Laemmli-Buffer

Incubate for 10 min, 95 °C

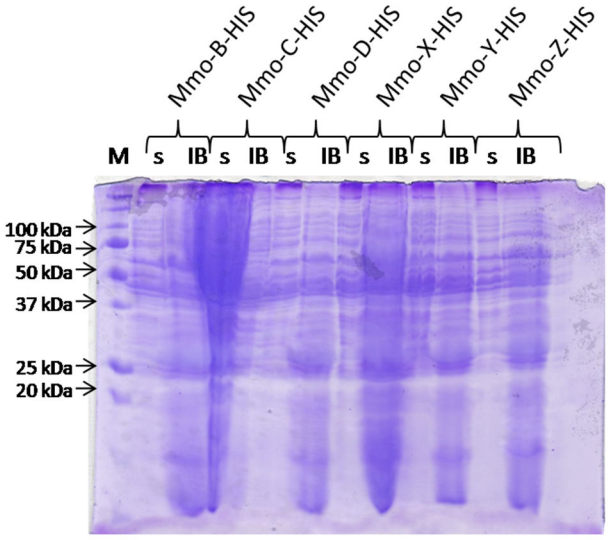


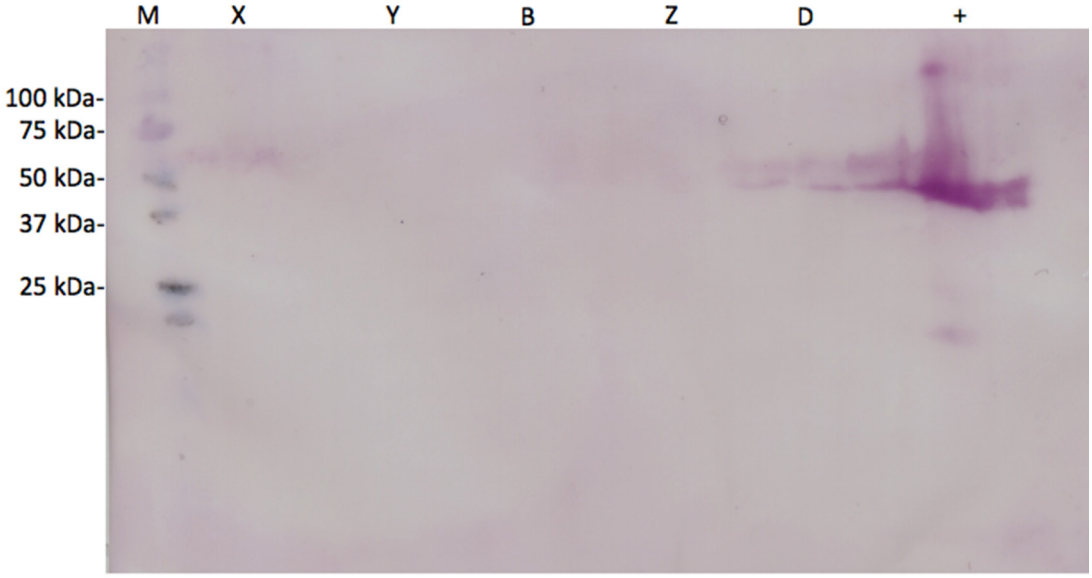
Fig. 2 SDS Page of all HIS-constructs both fro soluble fraction (s) and inclusion bodies (IB)

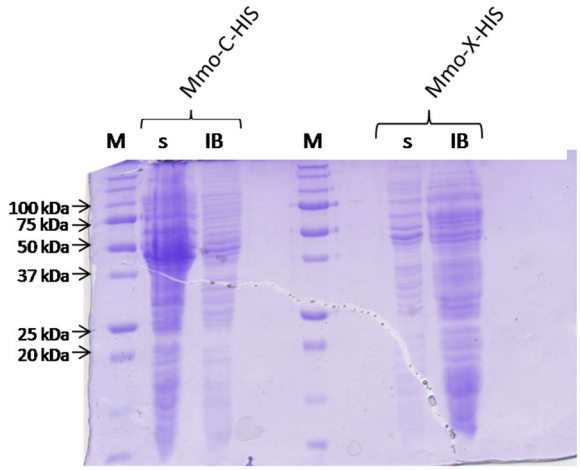
15.08.2014

Maren, Rüdiger, Oli, Anna

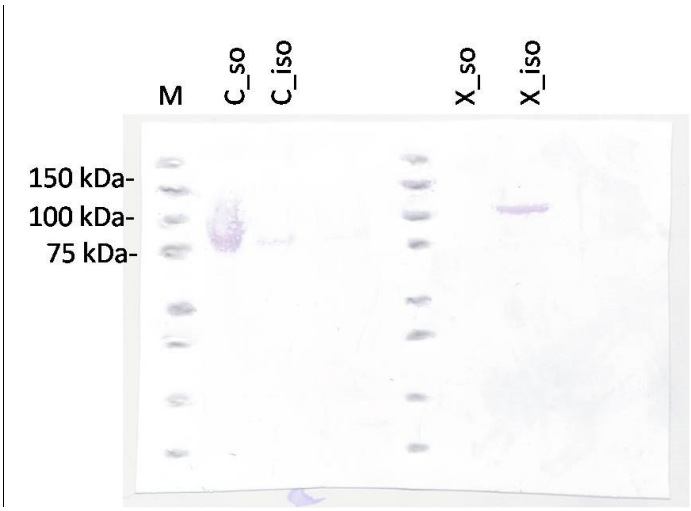
Western blot and immuno stain of SDS-PAGE from yesterday. Samples: all mmo-his constructs, both the soluble and inclusion bodies.

SDS-Page: 4 µl of construct mmo-C and mmo-X (Inclusion and soluble fraction) for validation





Blot result:



→ C is partly synthesized in soluble fraction and in inclusion bodies

X is completely synthesized in inclusion bodies.

Niels, Rüdiger

02.09.2014

Transformation

Of all His-construct in vector pSB1A3 in JM 109 cells with chaperones C1, C2 and C3.

→ Plating and cultivation over night.

Niels, Rüdiger

03.09.2014

Colonies grew on all plates. Precultures are inoculated and cultivated over night in 5 mL 2YT (Cm+Amp). Additionally , XL1 Blue is inoculated in 5 ml 2YT (Tet).

Niels, Oli, Rüdiger

04.09.2014

OD of precultures is measured and maincultures with a volume of 50 mL are inoculated to OD 0,1. Chaperones are induced at this point. The maincultures are cultivated to OD ≈0,5. Then the His-constructs are induced with 5 µL IPTG per culture. The temperature is set from 37 °C to 30 °C. OD is now measured every 2 hours. Additionally, every 2 h 1 mL of an Arabinose solution of ~0,1/mL is added to every culture. 6 h after induction with IPTG 25 mL are taken from the culture, pelletized and stored at -20 °C.

Nils, Oli, Rüdiger

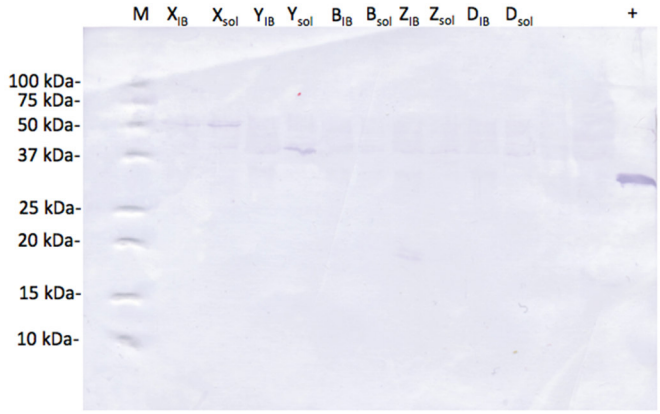
05.09.2014

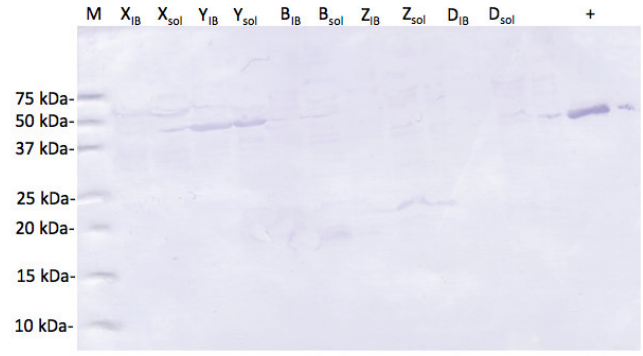
20 h after induction with IPTG OD is measured and the rest of the culture is pelletized and stored at -20 °C

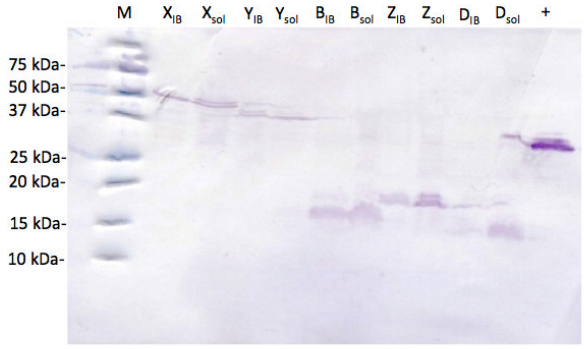
6.9.14

Maren Niels

Blot and Immunostain of the pelletized bacterial cultures (4.9.14) see figure 1-3







→ After coexpression with chaperon C2 all subunits can be found in soluble fraction (see Fig. 3)